

AC/TMH:gth 09/07/06 577617 AG03-071C  
PATENTAttorney Reference Number 6616-72707-02  
Application Number 10/697,787

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In re application of: Federspiel *et al.*****Application No. 10/697,787****Filed: October 29, 2003****Confirmation No. 1171****For: GENERATION OF PLANTS WITH  
IMPROVED PATHOGEN RESISTANCE  
AND DROUGHT TOLERANCE****FILED VIA EFS****Examiner: Medina Ahmed Ibrahim****Art Unit: 1638****Attorney Reference No. 6616-72707-02****SUBMITTED VIA ELECTRONIC FILING SYSTEM  
UNITED STATES PATENT AND TRADEMARK OFFICE****DECLARATION UNDER 37 CFR §1.132**

1. I, Dr. John P. Davies, hold the position of Director II, Plant Trait Discovery, at Exelixis Plant Sciences, Portland, Oregon. I have a Ph.D. in Molecular Biology and have 24 years of experience working in the field of plant physiology. I have performed or supervised the experiments described herein, which are an extension of the work described in the above-referenced application (U.S. Patent Application No. 10/697,787; hereinafter the '787 application).

2. I have participated in experiments demonstrating that certain orthologs of the polypeptide PRDT1 (SEQ ID NO: 2) confer both pathogen resistance and drought tolerance. Four of these orthologs are explicitly disclosed in Example 4 of the '787 application (SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14) and supplemental data related to their pathogen resistance and drought tolerance phenotypes are presented herein, along with supplemental data for SEQ ID NO: 2. Data related to a fifth ortholog (referred to herein as "At4g36570") are also presented herein. At4g36570 is a DNA binding/transcription factor of *Arabidopsis thaliana* (GenBank Accession No. NP\_195375.1) and has a SANT domain from residues 8 to 64.

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3. In order to characterize PRDT1 orthologs that confer pathogen resistance, the coding sequences of the PRDT1 homologous genes encoding SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, and At4g36570 were cloned behind the strong constitutive CsVMV promoter and transformed into wild-type Col-0 *Arabidopsis thaliana* plants, as taught in the specification at Example 6. Primary transformants (T1 plants) were selected and allowed to go to seed. Seed from these plants were planted and evaluated for resistance to *Peronospora parasitica* as follows: In separate experiments for each PRDT1 homolog, approximately 75 to 100 individual seedlings from different primary transformants were planted, stratified and grown for 1 week in a growth chamber. The one week old seedlings were inoculated with *Peronospora parasitica*. After one week, the seedlings were scored for *Peronospora parasitica* infection by counting the number of conidophores on a cotyledon. Plants with no conidiophores on a cotyledon were scored 0, plants with 1-5 were scored 1, plants with 6-10 were scored 2, plants with 10-20 were scored 3 and plants with more than 20 conidophores on a cotyledon were scored 4. Wild-type Col-0 seedlings were included as a control.

4. Fourteen lines over-expressing SEQ ID NO: 14 were examined. None of these lines were significantly more resistant to *Peronospora parasitica* than wild-type Col-0 plants (Exhibit A). Ten lines over-expressing SEQ ID NO: 11 were examined. Five of these lines were significantly more resistant to *Peronospora parasitica* than wild-type Col-0 plants; in these lines 31% to 65% of the transgenic plants were scored 0 or 1 while in wild-type Col-0 plants only 2% of the plants scored 0 or 1 (Exhibit B). Twelve lines over-expressing SEQ ID NO: 12 were examined. Two of these lines were significantly more resistant to *Peronospora parasitica* than wild-type Col-0 plants; in these lines 32% and 56%, respectively, of the transgenic plants scored 0 or 1 while in wild-type Col-0 control plants only 3% and 1%, respectively, of the plants scored 0 or 1 (Exhibit C). Ten lines over-expressing SEQ ID NO: 13 were examined. None of these lines were significantly more resistant to *Peronospora parasitica* than wild-type Col-0 plants (Exhibit D). Nineteen lines over-expressing At4g36570 were examined. Eight of these lines were significantly more resistant to *Peronospora parasitica* than wild-type Col-0 plants; in these lines 36 to 100% of the transgenic plants were scored 0 or 1 while in wild-type Col-0 control plants 18%, of the plants scored 0 or 1 (Exhibit E). Thus, over-expression of SEQ ID NO: 11,

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SEQ ID NO: 12, and At4g36570 confers resistance to *Peronospora parasitica* (see also Paragraph 9, below).

5. Supplemental pathogen resistance data from PRDT1 (SEQ ID NO: 2) was also gathered. Forty-eight lines over-expressing SEQ ID NO: 2 were examined. Thirty-two of these lines were significantly more resistant to *Peronospora parasitica* than wild-type Col-0 plants; in these lines, 40 to 100% of the transgenic plants were scored 0 or 1 while 0 to 16% of the control plants scored 0 or 1 (Exhibit F). These results supplement and further support the *Peronospora parasitica*-resistance data presented in the specification at Examples 2, 5 and 6.

6. In order to characterize PRDT1 orthologs that confer drought tolerance, plants containing activation tagging (ACTTAG) elements near the PRDT1 orthologous genes encoding SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and At4g36570 were identified, using methods as in the specification at Example 8. Primary transformants (T1 plants) were selected and allowed to go to seed. Seed from these plants were planted and evaluated for drought tolerance as follows: Approximately 18 seeds from T2 populations were planted in soil in 4 inch pots. The seed were stratified at 4° C for 3 days and then grown in the growth room for 4 weeks. Drought stress was imposed on the plants by withholding water 28 days after transfer to the growth room, at this time the plants were beginning to bolt. Observations on every individual plant in each pot were made after the last watering (day 0), seven days after the last watering (day 7), fourteen days after the last watering (day 14) and seventeen days after the last watering (day 17). Plants that were fully turgid were scored 0, plants that showed slight wilting were scored 1, plants that showed significant wilting were scored 2 and plants that were fully desiccated were scored 3.

7. The lines W000092158 and W000148331 contain ACTTAG elements within 10 kbp of the sequence encoding SEQ ID NO: 11 and were tested for a drought tolerant phenotype. After seventeen days without water, 72% of the Col-0 plants were fully desiccated. However, 100% of W000092158 and 58% of W000148331 plants were either fully turgid or slightly wilting, indicating that they are more tolerant of drought conditions than the wild-type Col-0 control plants (Exhibit B). The lines W000171162 and W000175277 contain ACTTAG elements within 10 kbp of the sequence encoding SEQ ID NO: 12 and were tested for a drought tolerant

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phenotype. However, neither of these lines showed an increase in drought tolerance compared with the wild-type Col-0 line (**Exhibit C**). The lines W000086832 and W000139673 contain ACTTAG elements within 10 kbp of the sequence encoding **SEQ ID NO: 13** and were tested for a drought tolerant phenotype. 72% of the Col-0 plants were fully desiccated. However, 54% of W000086832 and 53% of W000139673 a plants were either fully turgid or slightly wilting, indicating that they are more tolerant of drought conditions than the wild-type Col-0 control plants (**Exhibit D**). The line W000025222 contains an ACTTAG element within 10 kbp of the sequence encoding **At4g36570** was tested for a drought tolerant phenotype. After seventeen days without water, 72% of the Col-0 plants were fully desiccated. However, 64% of W000025222 a plants were either fully turgid or slightly wilting, indicating that they are more tolerant of drought conditions than the wild-type Col-0 control plants (**Exhibit E**). Thus, over-expression of **SEQ ID NO: 11**, **SEQ ID NO: 13**, and **At4g36570** confers resistance to drought tolerance (see also Paragraph 9, below).

8. Supplemental drought tolerance data from PRDT1 (**SEQ ID NO: 2**) was also gathered. Experiments using ACTTAG lines containing inserts near PRDT1 were performed. The lines W000114956 and W000091083 contain inserts within 10 kbp of the sequence encoding **SEQ ID NO: 2**. After seventeen days without water, 72% of the Col-0 plants were fully desiccated. However, 73% of W000114956 and 61% of W000091083 plants were either fully turgid or slightly wilting, indicating that they are more tolerant of drought conditions than the wild-type Col-0 control plants (**Exhibit F**). Transgenic lines over-expressing PRDT1 were also tested in a drought tolerance experiment. After seventeen days without water 72% of the Col-0 plants were fully desiccated. However, between 53% and 100% of the transgenic plants over-expressing **SEQ ID NO: 2** from the CsVMV promoter were either fully turgid or slightly wilting, indicating that they are more tolerant of drought conditions than the wild-type Col-0 control plants (**Exhibit F**). These results provide additional support for the drought tolerance data presented in the specification at Examples 3, 7 and 8.

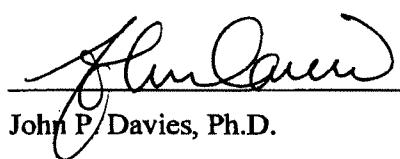
9. The data characterizing the pathogen resistance and drought tolerance of PRDT1 and the PRDT1 orthologs are summarized in the table below. "Y" indicates that the gene conferred pathogen resistance or drought tolerance when directly over-expressed in a transgenic line or in

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an ACTTAG line; "N" indicates that that the gene did not confer pathogen resistance or drought tolerance. Of six genes tested (PRDT1 and five orthologs), five genes conferred resistance to drought tolerance or pathogen resistance and of these, three conferred resistance to both phenotypes.

	Pathogen Resistance	Drought Tolerance
SEQ ID NO: 14	N	Not tested
SEQ ID NO: 11	Y	Y
SEQ ID NO: 2	Y	Y
SEQ ID NO: 13	N	Y
SEQ ID NO: 12	Y	N
At4g36570	Y	Y

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

  
John P. Davies, Ph.D.September 7, 2006  
Date

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## Exhibit A

## SEQ ID NO: 14 Pathogen Resistance Data

Flat	T1 Name	T1 Parent ID	TAIR Gene	Actual Counts						Percentage of Plants Examined						
				0	1	2	3	4	No Score	Total	0	1	2	3	4	0+1
PF20001165	COL-0			8	2	2	113	69	0	194	4%	1%	1%	58%	36%	5%
PF20001165	DX01348001	ZX00109SP1	At1g19490	1	0	1	14	53	0	69	1%	0%	1%	20%	77%	1%
PF20001165	DX01348002	ZX00109SP1	At1g19490	2	0	1	43	57	0	103	2%	0%	1%	42%	55%	2%
PF20001165	DX01348003	ZX00109SP1	At1g19490	5	2	0	13	48	0	68	7%	3%	0%	19%	71%	10%
PF20001165	DX01348004	ZX00109SP1	At1g19490	0	0	1	25	52	0	78	0%	0%	1%	32%	67%	0%
PF20001165	DX01348005	ZX00109SP1	At1g19490	0	0	0	6	44	0	50	0%	0%	0%	12%	88%	0%
PF20001165	DX01348007	ZX00109SP1	At1g19490	0	2	2	8	59	0	71	0%	3%	3%	11%	83%	3%
PF20001165	DX01348008	ZX00109SP1	At1g19490	0	0	2	27	53	0	82	0%	0%	2%	33%	65%	0%
PF20001165	DX01348009	ZX00109SP1	At1g19490	0	0	0	12	57	0	69	0%	0%	0%	17%	83%	0%
PF20001165	DX01348010	ZX00109SP1	At1g19490	1	0	0	12	69	0	82	1%	0%	0%	15%	84%	1%
PF20001165	DX01348011	ZX00109SP1	At1g19490	2	0	0	35	37	0	74	3%	0%	0%	47%	50%	3%
PF20001166	COL-0			6	0	7	73	113	0	199	3%	0%	4%	37%	57%	3%
PF20001166	DX01348012	ZX00109SP1	At1g19490	1	0	4	23	23	0	51	2%	0%	8%	45%	45%	2%
PF20001166	DX01348013	ZX00109SP1	At1g19490	0	0	0	12	50	0	62	0%	0%	0%	19%	81%	0%
PF20001166	DX01348014	ZX00109SP1	At1g19490	0	0	0	9	53	0	62	0%	0%	0%	15%	85%	0%
PF20001166	DX01348015	ZX00109SP1	At1g19490	1	1	3	10	45	0	60	2%	2%	5%	17%	75%	3%

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Flat	T1 Name	T1 Parent ID	TAIR Gene	Actual Counts						Percentage of Plants Examined						
				0	1	2	3	4	No Score	Total	0	1	2	3	4	0+1
PF20001164	COL-0			2	2	9	119	57	0	189	1%	1%	5%	63%	30%	2%
PF20001164	DX01349001	ZX00110SP1	At1g19510	1	4	6	40	21	0	72	1%	8%	58%	20%	7%	
PF20001164	DX01349002	ZX00110SP1	At1g19510	27	23	21	25	6	0	102	26%	23%	21%	25%	6%	49%
PF20001164	DX01349006	ZX00110SP1	At1g19510	2	9	14	53	13	0	91	2%	10%	15%	58%	14%	12%
PF20001164	DX01349008	ZX00110SP1	At1g19510	39	21	10	18	5	0	93	42%	23%	11%	19%	5%	85%
PF20001164	DX01349009	ZX00110SP1	At1g19510	0	0	2	45	11	0	58	0%	0%	3%	78%	19%	0%
PF20001164	DX01349010	ZX00110SP1	At1g19510	18	21	11	30	0	0	78	21%	27%	14%	38%	0%	47%
PF20001164	DX01349014	ZX00110SP1	At1g19510	12	11	16	34	1	0	74	16%	15%	22%	46%	1%	31%
PF20001164	DX01349015	ZX00110SP1	At1g19510	15	24	22	15	0	0	76	20%	32%	29%	20%	0%	51%
PF20001164	ZX01018001	ZX00110SP1	At1g19510	1	6	13	48	5	0	73	1%	8%	18%	66%	7%	10%
PF20001164	ZX01018002	ZX00110SP1	At1g19510	2	4	17	31	2	1	57	4%	7%	30%	54%	4%	11%

**SEQ ID NO: 11 Drought Tolerance Data**

Line	DRO	TAIR	Pool ID	parent ID	drought score (DAY17)										
					0	1	2	3	0+1	Total	0	1	2	3	0+1
W000092158	DRO403	At1g19510	Z002050	W000092158	12	0	0	0	12	12	100.0%	0.0%	0.0%	0.0%	100.0%
W000148331	DRO403	At1g19510	Z002083	W000148331	1	10	7	1	11	19	5.3%	52.6%	36.8%	5.3%	57.9%
Col-0			all Col-0	drought	1	21	107	327	22	456	0.2%	4.8%	23.5%	71.7%	4.8%

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Flat	T1 Name	T1 Parent ID	TAIR Gene	Actual Counts						Percentage of Plants Examined						
				0	1	2	3	4	No Score	Total	0	1	2	3	4	0+1
	COL-0			6	0	7	73	113	0	199	3%	0%	4%	37%	57%	3%
PF20001166	ZX01018003	ZX00049SP1	At2g21650	1	5	14	22	25	0	67	1%	7%	21%	33%	37%	9%
PF20001166	ZX01018005	ZX00049SP1	At2g21650	5	8	27	23	21	0	84	6%	10%	32%	27%	25%	15%
PF20001166	ZX01018006	ZX00049SP1	At2g21650	3	0	0	42	39	0	84	4%	0%	0%	50%	46%	4%
PF20001166	ZX01018007	ZX00049SP1	At2g21650	2	5	19	43	5	0	74	3%	7%	28%	58%	7%	9%
PF20001166	ZX01018008	ZX00049SP1	At2g21650	13	10	20	29	1	0	73	18%	14%	27%	40%	1%	32%
PF20001166	ZX01018009	ZX00049SP1	At2g21650	4	12	23	40	5	0	84	5%	14%	27%	48%	6%	19%
PF20001167	COL-0			1	0	2	89	31	0	123	1%	0%	2%	72%	25%	1%
PF20001167	ZX01018010	ZX00049SP1	At2g21650	0	2	24	71	7	0	104	0%	2%	23%	68%	7%	2%
PF20001167	ZX01018011	ZX00049SP1	At2g21650	3	8	17	49	2	0	79	4%	10%	22%	62%	3%	14%
PF20001167	ZX01018012	ZX00049SP1	At2g21650	1	3	9	33	39	0	85	1%	4%	11%	39%	46%	5%
PF20001167	ZX01018013	ZX00049SP1	At2g21650	26	23	14	12	12	0	87	30%	26%	16%	14%	14%	56%
PF20001167	ZX01018014	ZX00049SP1	At2g21650	0	0	5	31	41	0	77	0%	0%	6%	40%	53%	0%
PF20001167	ZX01018015	ZX00049SP1	At2g21650	0	0	4	23	55	0	82	0%	0%	5%	28%	67%	0%

**SEQ ID NO: 12 Drought Tolerance Data**

Line	DRO	TAIR	Pool ID	parent ID	drought score (DAY17)										
					0	1	2	3	0+1	total	0	1	2	3	0+1
W000171162	DRO401	At2g21650	Z002086	W000171162	0	3	10	6	3	19	0.0%	15.8%	52.6%	31.6%	15.8%
W000175277	DRO401	At2g21650	Z002095	W000175277	0	0	21	9	0	30	0.0%	0.0%	70.0%	30.0%	0.0%
Col-0			all Col-0	drought	1	21	107	327	22	456	0.2%	4.6%	23.5%	71.7%	4.8%

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Flat	T1 Name	T1 Parent ID	TAIR Gene	Actual Counts							Percentage of Plants Examined						
				0	1	2	3	4	No Score	Total	0	1	2	3	4	0+1	
PF20001163	COL-0			1	0	17	164	7	0	189	1%	0%	9%	87%	4%	1%	
PF20001163	DX01157008	ZX00097SP1	At4g39250	0	10	27	39	1	0	77	0%	13%	35%	51%	1%	13%	
PF20001163	DX01157007	ZX00097SP1	At4g39250	2	2	13	45	0	0	62	3%	3%	21%	73%	0%	6%	
PF20001163	DX01157008	ZX00097SP1	At4g39250	0	4	18	39	0	0	59	0%	7%	27%	66%	0%	7%	
PF20001163	DX01157009	ZX00097SP1	At4g39250	1	3	13	38	0	0	55	2%	5%	24%	69%	0%	7%	
PF20001163	DX01157010	ZX00097SP1	At4g39250	0	0	3	43	12	0	58	0%	0%	5%	74%	21%	0%	
PF20001163	DX01157011	ZX00097SP1	At4g39250	0	0	18	40	0	0	58	0%	0%	31%	69%	0%	0%	
PF20001163	DX01157012	ZX00097SP1	At4g39250	4	0	10	48	0	0	62	6%	0%	16%	77%	0%	6%	
PF20001163	DX01157013	ZX00097SP1	At4g39250	0	0	6	51	0	0	57	0%	0%	11%	89%	0%	0%	
PF20001163	DX01157014	ZX00097SP1	At4g39250	1	0	6	48	0	1	58	2%	0%	11%	86%	0%	2%	
PF20001163	DX01157015	ZX00097SP1	At4g39250	4	3	15	39	0	0	61	7%	5%	25%	64%	0%	11%	

**SEQ ID NO: 13 Drought Tolerance Data**

Line	DRO	TAIR	Pool ID	parent ID	drought score (DAY17)											
					0	1	2	3	0+1	Total	0	1	2	3	0+1	
W000139673	DRO402	At4g39250	Z002077	W000139673	6	1	5	1	7	13	46.2%	7.7%	38.5%	7.7%	53.8%	
W000086832	DRO402	At4g39250	Z002044	W000086832	0	9	3	5	9	17	0.0%	52.9%	17.6%	29.4%	52.9%	
Col-0				all Col-0	drought	1	21	107	327	22	456	0.2%	4.6%	23.5%	71.7%	4.8%

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Flat	T1 Name	T1 Parent ID	TAIR Gene	Actual Counts							Percentage of Plants Examined						
				0	1	2	3	4	No Score	Total	0	1	2	3	4	0+1	
PF20001162	COL-0			0	54	60	177	12	0	303	0%	18%	20%	58%	4%	18%	
PF20001162	DX01156011	ZX00096SP1	At4g36570	0	0	10	46	0	0	56	0%	0%	18%	82%	0%	0%	
PF20001162	DX01156012	ZX00096SP1	At4g36570	0	0	4	51	0	0	55	0%	0%	7%	83%	0%	0%	
PF20001162	DX01156013	ZX00096SP1	At4g36570	1	1	12	43	2	0	59	2%	2%	20%	73%	3%	3%	
PF20001162	DX01156014	ZX00096SP1	At4g36570	0	0	29	29	0	0	58	0%	0%	50%	50%	0%	0%	
PF20001162	DX01156015	ZX00096SP1	At4g36570	7	21	33	0	0	0	61	11%	34%	54%	0%	0%	46%	
PF20001162	DX01157004	ZX00096SP1	At4g36570	19	19	18	5	0	0	69	32%	32%	27%	8%	0%	64%	
PF20001162	DX01157002	ZX00096SP1	At4g36570	28	31	6	0	0	0	65	43%	48%	8%	0%	0%	91%	
PF20001162	DX01157003	ZX00096SP1	At4g36570	26	28	0	0	0	0	54	48%	52%	0%	0%	0%	100%	
PF20001162	DX01157004	ZX00096SP1	At4g36570	11	34	18	0	0	0	63	17%	54%	29%	0%	0%	71%	
PF20001162	DX01157005	ZX00096SP1	At4g36570	23	35	9	0	0	0	67	34%	52%	13%	0%	0%	87%	
PF20001163	COL-0			17	16	25	96	33	0	187	9%	9%	13%	51%	18%	18%	
PF20001163	DX01156001	ZX00096SP1	At4g36570	12	15	18	31	1	0	75	16%	20%	21%	41%	1%	36%	
PF20001163	DX01156002	ZX00096SP1	At4g36570	8	1	14	72	1	0	96	8%	1%	15%	75%	1%	9%	
PF20001163	DX01156003	ZX00096SP1	At4g36570	6	35	24	8	1	0	74	8%	47%	32%	11%	1%	55%	
PF20001163	DX01156004	ZX00096SP1	At4g36570	2	3	9	54	1	0	69	3%	4%	13%	78%	1%	7%	
PF20001163	DX01156005	ZX00096SP1	At4g36570	3	0	8	48	0	0	59	5%	0%	14%	81%	0%	5%	
PF20001163	DX01156006	ZX00096SP1	At4g36570	4	0	1	52	0	0	57	7%	0%	2%	91%	0%	7%	
PF20001163	DX01156007	ZX00096SP1	At4g36570	0	0	3	49	3	0	55	0%	0%	5%	89%	5%	0%	
PF20001163	DX01156008	ZX00096SP1	At4g36570	12	2	8	58	0	1	79	15%	3%	8%	73%	0%	18%	
PF20001163	DX01156009	ZX00096SP1	At4g36570	8	0	3	61	1	0	73	11%	0%	4%	84%	1%	11%	

**At4g36570 Drought Tolerance Data**

Line	DRO	TAIR	Pool ID	parent ID	drought score (DAY17)										
					0	1	2	3	0+1	total	0	1	2	3	0+1
W000025222	DRO404	At4g36570	Z002029	W000025222	6	3	3	2	9	14	42.9%	21.4%	21.4%	14.3%	64.3%
Col-0			all Col-0	drought	1	21	107	327	22	456	0.2%	4.6%	23.5%	71.7%	4.6%

AC/TMH:gth 09/07/06 577617 AG03-071C  
PATENTAttorney Reference Number 6616-72707-02  
Application Number 10/697,787

## Exhibit F

## SEQ ID NO: 2 Pathogen Resistance Data

Experiment	Flat	T1 Name	T1 Parent ID	TAIR Gene	Actual Counts						Percentage of Plants Examined						
					0	1	2	3	4	No Score	Total	0	1	2	3	4	0+1
1	PF20001059	Z000643001	Z000186SP1	At1g75250	14	1	1	76	13		105	13.3%	1.0%	1.0%	72.4%	12.4%	14.3%
	PF20001059	Z000643002	Z000186SP1	At1g75250	10	5	32	4			51	19.6%	0.0%	9.8%	62.7%	7.8%	19.8%
	PF20001059	Z000643003	Z000186SP1	At1g75250	12	3	13	22	4		54	22.2%	5.6%	24.1%	40.7%	7.4%	27.8%
	PF20001059	Z000643005	Z000186SP1	At1g75250	16	2	14	10			42	38.1%	4.8%	33.3%	23.8%	0.0%	42.9%
	PF20001059	Z000643007	Z000186SP1	At1g75250	11	8	14	21			54	20.4%	14.8%	25.9%	38.9%	0.0%	36.2%
	PF20001059	Z000643008	Z000186SP1	At1g75250	25	2	8	5			40	62.5%	5.0%	20.0%	12.5%	0.0%	67.5%
	PF20001059	Z000643009	Z000186SP1	At1g75250	14	9	10	23			56	26.0%	18.1%	17.9%	41.1%	0.0%	41.1%
	PF20001059	Z000643011	Z000186SP1	At1g75250	22	2	11	11			46	47.8%	4.3%	23.9%	23.9%	0.0%	52.2%
	PF20001059	Z000643012	Z000186SP1	At1g75250	16	3	8	25	4		54	28.6%	5.6%	11.1%	46.3%	7.4%	35.2%
	PF20001059	Z000643013	Z000186SP1	At1g75250	12	1	6	12	3		33	38.4%	3.0%	15.2%	38.4%	9.1%	39.4%
	PF20001059	COL-0	(blank)	COL-0	8	22	36	94	39		199	4.0%	11.1%	18.1%	47.2%	19.6%	15.1%
2	PF20001060	Z000643021	Z000186SP1	At1g75250	28	4	8	6	3		49	57.1%	8.2%	16.3%	12.2%	6.1%	65.3%
	PF20001060	Z000643022	Z000186SP1	At1g75250	3	1	2	37	8		51	5.9%	2.0%	3.9%	72.5%	15.7%	7.8%
	PF20001060	Z000643023	Z000186SP1	At1g75250	29	2	6	12	1		60	58.0%	4.0%	12.0%	24.0%	2.0%	62.0%
	PF20001060	Z000643024	Z000186SP1	At1g75250	7			9			16	43.8%	0.0%	0.0%	58.3%	0.0%	43.8%
	PF20001060	Z000643062	Z000186SP1	At1g75250	41	1		8	2		52	78.8%	1.6%	0.0%	15.4%	3.8%	80.8%
	PF20001060	COL-0	(blank)	COL-0	22	11	34	100	34		201	10.9%	5.5%	16.9%	49.8%	16.9%	16.4%
3	PF20001064	Z000645058	Z000190SP1	At1g75250	1	2	10	9			22	0.0%	4.5%	9.1%	45.5%	40.9%	4.5%
	PF20001064	Z000645055	Z000190SP1	At1g75250	3	3	11	4			21	0.0%	14.3%	14.3%	52.4%	19.0%	14.3%
4	PF20001064	COL-0	(blank)	COL-0	1	7	5				13	0.0%	0.0%	7.7%	53.8%	38.5%	0.0%
	PF20001064	Z000645056	Z000190SP1	At1g75250	4	4	16	2			26	0.0%	15.4%	15.4%	61.5%	7.7%	15.4%
5	PF20001064	Z000645057	Z000190SP1	At1g75250	1	7	4	8	1		21	4.8%	33.3%	19.0%	38.1%	4.8%	38.1%
	PF20001064	COL-0	(blank)	COL-0	1	4	6	2			12	0.0%	6.3%	33.3%	41.7%	16.7%	8.3%
	PF20001113	Z000643008	Z000186SP1	At1g75250	4	1					5	80.0%	20.0%	0.0%	0.0%	0.0%	100.0%
	PF20001113	Z000643011	Z000186SP1	At1g75250	6	1					8	75.0%	12.5%	12.5%	0.0%	0.0%	87.5%
	PF20001113	Z000643014	Z000186SP1	At1g75250	6						8	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
	PF20001113	Z000643015	Z000186SP1	At1g75250	5	4					9	55.6%	44.4%	0.0%	0.0%	0.0%	100.0%
	PF20001113	Z000643019	Z000186SP1	At1g75250	1		1				2	50.0%	0.0%	50.0%	0.0%	0.0%	50.0%
	PF20001113	Z000643021	Z000186SP1	At1g75250	1		6	1			8	12.5%	0.0%	0.0%	75.0%	12.5%	12.5%
	PF20001113	Z000643022	Z000186SP1	At1g75250	4	1	3	2	1		11	36.4%	9.1%	27.3%	18.2%	9.1%	46.5%
6	PF20001113	Z000643023	Z000186SP1	At1g75250	2		3	3			8	0.0%	25.0%	37.5%	37.5%	0.0%	25.0%
	PF20001113	Z000643062	Z000186SP1	At1g75250	3	1					4	76.0%	25.0%	0.0%	0.0%	0.0%	100.0%
	PF20001113	COL-0	(blank)	COL-0	1	3	2	24	22		52	1.9%	5.8%	3.8%	46.2%	42.3%	7.7%
	PF20001118	Z000643008	Z000186SP1	At1g75250	3	2					5	60.0%	40.0%	0.0%	0.0%	0.0%	100.0%
	PF20001118	Z000643011	Z000186SP1	At1g75250	5	3		3			11	45.5%	27.3%	0.0%	0.0%	27.3%	72.7%
7	PF20001118	Z000643014	Z000186SP1	At1g75250	2	2		1			5	40.0%	40.0%	0.0%	20.0%	0.0%	80.0%
	PF20001118	Z000643015	Z000186SP1	At1g75250	5	1	1				7	71.4%	14.3%	14.3%	0.0%	0.0%	85.7%
	PF20001118	Z000643019	Z000186SP1	At1g75250	6	3					11	72.7%	27.3%	0.0%	0.0%	0.0%	100.0%
	PF20001118	Z000643021	Z000186SP1	At1g75250	5	1		6			12	41.7%	8.3%	0.0%	0.0%	50.0%	50.0%
	PF20001118	Z000643022	Z000186SP1	At1g75250	1	4	4	2			11	9.1%	0.0%	38.4%	38.4%	18.2%	9.1%
8	PF20001118	Z000643023	Z000186SP1	At1g75250	1	1	3	6	1		11	9.1%	9.1%	27.3%	45.5%	9.1%	18.2%
	PF20001118	Z000643062	Z000186SP1	At1g75250	2	1					3	88.7%	33.3%	0.0%	0.0%	0.0%	100.0%
	PF20001118	COL-0	(blank)	COL-0	2	2	16	36			56	0.0%	3.8%	3.8%	28.6%	84.3%	3.6%
	PF20001119	Z000643008	Z000186SP1	At1g75250	3	1	1	1	1		7	42.9%	14.3%	14.3%	14.3%	14.3%	57.1%
	PF20001119	Z000643011	Z000186SP1	At1g75250	4	4	2				10	0.0%	40.0%	40.0%	20.0%	0.0%	40.0%
9	PF20001119	Z000643014	Z000186SP1	At1g75250	2	2	3	2	3		12	16.7%	16.7%	25.0%	16.7%	25.0%	33.3%
	PF20001119	Z000643015	Z000186SP1	At1g75250	2	1	3	2	2		8	25.0%	12.5%	37.5%	25.0%	0.0%	37.5%
	PF20001119	Z000643019	Z000186SP1	At1g75250	6	3					8	62.5%	37.5%	0.0%	0.0%	0.0%	100.0%
	PF20001119	Z000643021	Z000186SP1	At1g75250	1	1	1	5			9	11.1%	11.1%	11.1%	11.1%	55.6%	22.2%
	PF20001119	Z000643022	Z000186SP1	At1g75250	2	3	6	3			14	14.3%	0.0%	21.4%	42.9%	21.4%	14.3%
10	PF20001119	Z000643023	Z000186SP1	At1g75250	3	3	6	1			12	0.0%	25.0%	0.0%	25.0%	50.0%	25.0%
	PF20001119	Z000643062	Z000186SP1	At1g75250	1	3	4	1			9	11.1%	33.3%	44.4%	11.1%	0.0%	44.4%
	PF20001119	COL-0	(blank)	COL-0	1		23	36			60	0.0%	0.0%	1.7%	38.3%	80.0%	0.0%
11	PF20001200	Z000643014	Z000186SP1	At1g75250	1		32				33	3.0%	0.0%	0.0%	97.0%	0.0%	3.0%
	PF20001200	COL-0	(blank)	COL-0	1		34				35	2.9%	0.0%	0.0%	97.1%	0.0%	2.9%
12	PF20001223	Z000643014	Z000186SP1	At1g75250	1		30				31	3.2%	0.0%	0.0%	96.8%	0.0%	3.2%
	PF20001223	COL-0	(blank)	COL-0	1		24				25	4.0%	0.0%	0.0%	96.0%	0.0%	4.0%

## SEQ ID NO: 2 Drought Tolerance Data

Line	DRO	TAIR	Relative treatment	Pool ID	parent ID	0	1	2	3	0+1	total	drought score (DAY17)						
												0	1	2	3	0+1		
W000114956	DRO4	At1g75250	29.18	drought	Z002062	W000114956	6	11	6	0	17	23	26.1%	47.8%	26.1%	0.0%	73.9%	
W000643083	DRO4	At1g75250	0.27	drought	Z002047	W000643083	4	7	7	0	11	18	22.2%	38.9%	38.9%	0.0%	61.1%	
Col-0				drought	all Col-0	drought	1	21	107	327	22	456	0.2%	4.8%	23.5%	71.7%	4.8%	

AC/TMH:gth 09/07/06 577617 AG03-071C  
PATENTAttorney Reference Number 6616-72707-02  
Application Number 10/697,787**Drought Tolerance Data for Transgenic Lines Over-Expressing SEQ ID NO: 2**

TAIR	T1 Plant ID	parent ID	drought scores (DAY 17)									
			0	1	2	3	0+1	total	0.0%	100.0%	200.0%	300.0%
A11g75250	Z000186SP1	Z000643014	30	3	0	0	33	33	90.9%	8.1%	0.0%	0.0%
A11g75250	Z000186SP1	Z000643026	2	0	0	0	2	2	100.0%	0.0%	0.0%	100.0%
A11g75250	Z000186SP1	Z000643012	18	6	0	1	22	23	88.6%	26.1%	0.0%	4.4%
A11g75250	Z000186SP1	Z000643021	1	11	1	0	12	13	7.7%	84.6%	7.7%	0.0%
A11g75250	Z000186SP1	Z000643015	15	11	2	3	26	31	48.4%	35.5%	6.5%	9.7%
A11g75250	Z000186SP1	Z000643011	5	4	3	0	8	12	41.7%	33.3%	25.0%	0.0%
A11g75250	Z000186SP1	Z000643009	7	1	4	3	8	15	48.7%	6.7%	26.7%	20.0%
	Col-0		1	21	107	327	22	458	0.2%	4.8%	23.5%	71.7%
												4.8%

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## The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIB

Current models of transcriptional co-activators and co-repressors envisage that these complexes function by protein-protein interaction mechanisms, although the details are not well understood<sup>1</sup>. Recently, a new co-repressor, N-CoR, was described as a regulator of thyroid and retinoic acid receptors<sup>2</sup>.

In self-comparisons of the N-CoR amino-acid sequence, we found two copies of a ~50-residue motif spaced 129 residues apart. These repeats are flanked by two regions that have repressor properties<sup>2</sup>. Sequence-database searches revealed one or two copies of this motif in several other proteins (Fig. 1a). Some of these are database entries of poorly determined function, but we noticed matches with three proteins from yeast that participate in basal or activated transcription complexes. SWI3 and ADA2 are components of either the SWI-SNF<sup>1,3</sup> or ADA<sup>1,4</sup> transcriptional activation complexes, while the B'' subunit of TFIIB<sup>5</sup> is a component of the RNA polymerase III initiation complex. Therefore, we call this the SANT (SWI3, ADA2, N-CoR and TFIIB B'') domain. Further refined database searches identified the I-SWI proteins (HuSN2L, CeSN2L, DmISWI and ScYBR245)<sup>6</sup> as a SANT-domain outgroup.

The coincidence between SANT-domain proteins with known functions and transcriptional co-factor initiation complexes suggests that the SANT domain is specifically involved in transcriptional regulation.

A clue to a putative function of SANT domains came from further sequence alignments. In both BLASTP and profile searches, SANT domains consistently scored well with the DNA-binding domains (DBDs) of myb-related proteins. The myb DBDs consist of two or three tandemly repeated subdomains, each resembling the homeo-domain helix-loop-helix motif<sup>7,8</sup>. SANT-myb DBD alignments show a number of highly conserved residues, including the three aromatic residues (Trp6, Trp26 and Trp49) important to the hydrophobic core of the myb repeats.

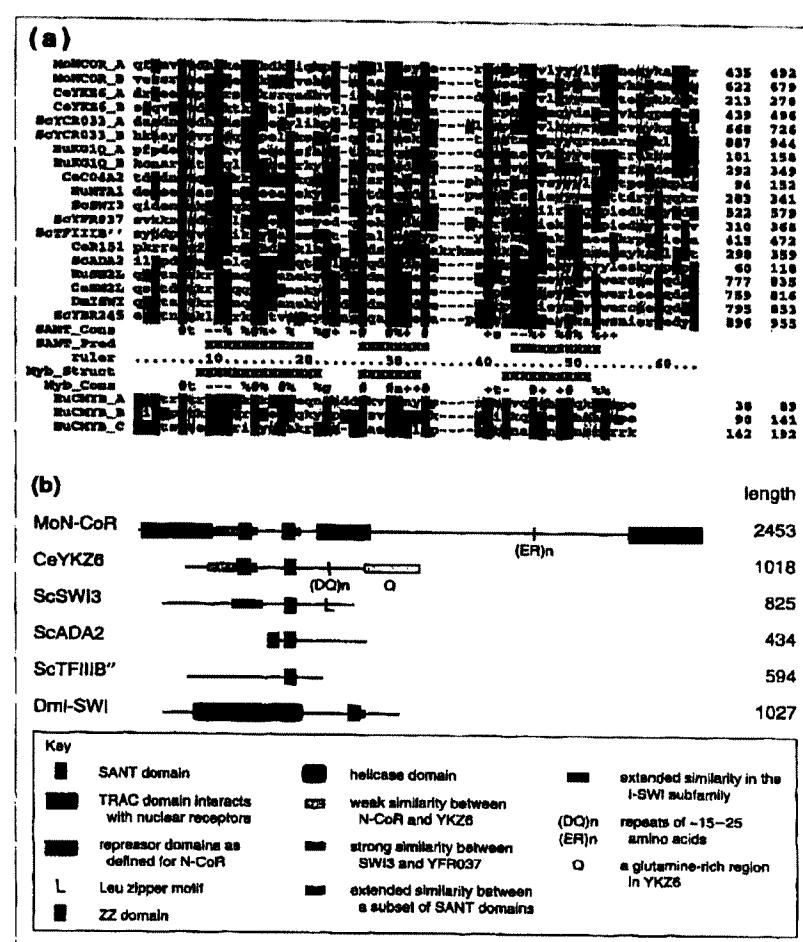
The similarity of SANT domains to myb DBDs is further supported by the SANT secondary structure prediction. The speculation that SANT domains are DBDs is also concordant with the frequent

presence of positive charges immediately carboxy-terminal to the third helix in positions that, based on the myb DBD-DNA complex<sup>9</sup>, would be well placed to interact with DNA phosphates.

As the minimal myb DBD is composed of two repeats, the DBD prediction is particularly strong for those proteins,

## PROTEIN SEQUENCE MOTIF

such as N-CoR, that display two SANT domains. For those proteins with one SANT domain, the implication is less clear. However, two proteins with single myb repeats implicated in DNA binding have been reported<sup>9,10</sup>. As the SWI-SNF and TFIIB complexes have considerable affinity for DNA, which is apparently not



**Figure 1.**  
**(a)** Alignment of SANT domains colour coded with the program COLOURMASK according to conserved residue similarity<sup>13</sup>. A consensus common to the SANT domains is shown: %, semi-conserved hydrophobicity; #, strongly conserved hydrophobicity; -, conserved acidic residues; +, conserved basic residues. A secondary structure prediction generated with the program PHD<sup>14</sup> is shown; H,  $\alpha$  helix; E, extended sheet. The three repeats of the human c-Myc-DNA-binding domain are likewise shown for comparison. For the myb repeats, the secondary structure assignment is based on the known three-dimensional structure of the murine c-Myc protein<sup>10</sup>. Original sequence database searches were performed with the BLASTP program<sup>15</sup> and subsequent profile-based database searches and pairwise comparisons were performed with WISETOOLS as previously described (Refs 13, 16; and World Wide Web (WWW) from <http://www.ocms.ox.ac.uk/~bfmey/wise/topwises.html>). The sequences used were (protein, database:identifier): MoNCoR, trn: MM35312\_1; CeYKZ6, sw: YKZ6\_CAEEL; ScYCR033, sw: YCS3\_YEAST; HuKG1Q, tr: HS0RFPKG1Q\_1; CeC04A2, tr: CEC04A2\_2; HuMTA1, trn: HS3511310\_1; ScSWI3, sw: SWI3\_YEAST; ScYFR037, sw: YPK7\_YEAST; ScTFIIB'', trn: SC31819\_1; CeR151, tr: CER151\_9; ScADA2, sw: ADA2\_yeast; HuSN2L, sw: SN2L\_HUMAN; CeSN2L, sw: SN2L\_CAEEL; DmISWI, tr: DMISWI\_1; ScYBR245, sw: YB95\_YEAST; HuCMYB, sw: MYB\_human. Abbreviations used: sw, SWISS-PROT; tr and trn, the translated versions of the EMBL and EMBLNEW databases (WWW from <http://www.embl-heidelberg.de/swi/wgetz7-info-trembl>). Species names are indicated as: Mo, mouse; Hu, human; Ce, *Caenorhabditis elegans*; Sc, *Saccharomyces cerevisiae*. The domain positions in the protein sequences are indicated at the right. Further information on the SANT domains and the methods used for sequence analysis are available on the WWW from <http://www.ulib.no/aastrand/SANT.html>. **(b)** Arrangement of SANT and other domains in a selected set of proteins.

# PROTEIN SEQUENCE MOTIF



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sequence specific, it is possible that single SANT domains contribute to general DNA affinity.

For TFIIB, site-specific DNA binding is directed by the recruitment factor, TFIIC, and can persist after TFIIC removal<sup>5</sup>. The identification of SANT domains in TFIIB, SWI-SNF, ADA and I-SWI complexes suggests, by analogy, that these co-activator complexes are specifically located to DNA sites by recruitment factors, whereupon they persist by virtue of their DNA-binding properties. Alternatively, DBDs are known to be common targets for protein-protein interactions<sup>11</sup> and the SANT domains might be adapted to this role.

The prediction that N-CoR has a DBD suggests that it might be a sequence-specific transcription factor. If so, then the relationship between N-CoR and both thyroid or retinoic acid receptors might be similar to the relationship between AP-1 and other nuclear receptors such as the glucocorticoid receptor<sup>12</sup>. In this case,

two signal-transduction pathways negatively regulate each other, apparently through protein-protein interactions between DNA-sequence-specific transcription factors.

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LOCUS AAF08790 1361 aa linear PLN 22-DEC-2000  
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 VERSION AAF08790.1 GI:6449046  
 DBSOURCE locus ATRPP5LE2 accession [AF180942.1](#)  
 KEYWORDS .  
 SOURCE *Arabidopsis thaliana* (thale cress)  
 ORGANISM *Arabidopsis thaliana*  
 Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons; rosids; eurosids II; Brassicales; Brassicaceae; *Arabidopsis*.  
 REFERENCE 1 (residues 1 to 1361)  
 AUTHORS Noel, L., Moores, T.L., van Der Biezen, E.A., Parniske, M., Daniels, M.J., Parker, J.E. and Jones, J.D.  
 TITLE Pronounced intraspecific haplotype divergence at the RPP5 complex disease resistance locus of *Arabidopsis*  
 JOURNAL *Plant Cell* 11 (11), 2099-2112 (1999)  
 PUBMED [10559437](#)  
 REFERENCE 2 (residues 1 to 1361)  
 AUTHORS Noel, L., Moores, T., van der Biezen, E.A., Parniske, M., Daniels, M.J., Parker, J.E. and Jones, J.D.G.  
 TITLE Direct Submission  
 JOURNAL Submitted (26-AUG-1999) Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich, Norfolk NR4 7UH, United Kingdom  
 COMMENT Method: conceptual translation.  
 FEATURES Location/Qualifiers  
 source 1..1361  
 /organism="Arabidopsis thaliana"  
 /db\_xref="taxon:3702"  
 /chromosome="4"  
 /map="C18a"  
 /ecotype="Landsberg erecta"  
 /note="4kb 5' of main contig with GenBank Accession Number AF180942"  
 Protein 1..1361  
 /product="downy mildew resistance protein RPP5"  
 Region 11..151  
 /region\_name="TIR"  
 /note="Toll - interleukin 1 - resistance; smart00255"  
 /db\_xref="CDD:47584"  
 Region 186..450  
 /region\_name="NB-ARC"

Exhibit H

```

/note="NB-ARC domain; pfam00931"
/db_xref="CDD:41006"
Region 212..335
/region_name="AAA"
/note="AAA-superfamily of ATPases associated with a wide
variety of cellular activities, including membrane fusion,
proteolysis, and DNA replication; cd00009"
/db_xref="CDD:28893"
Region <901..>1116
/region_name="COG4886"
/note="Leucine-rich repeat (LRR) protein [Function
unknown]; COG4886"
/db_xref="CDD:34495"
CDS 1..1361
/gene="RPP5"
/coded_by="join(AF180942.1:77312..77790,
AF180942.1:77917..78991,AF180942.1:79181..79474,
AF180942.1:79581..79994,AF180942.1:80101..80598,
AF180942.1:80811..81842,AF180942.1:81970..82180,
AF180942.1:82319..82401)"

```

## ORIGIN

```

1 maassssgrr rydvfpsfsg vdvrktflsh llkaldgksi ntfidhgier srtiapelis
61 airearisiv ifsknyasst wclnelveih kcfndlqgmv ipvfydvdps evrkqtgefg
121 kfektcevs kdkqpgdqkq rwvqaltdia niagedllng pneahmveki sndvsnkli
181 rskcfddfvg ieahieaiks vlcleskear mvgiwgqsgsi gkstigralf sqlssqfhhr
241 aflytkstsg sdvsgmklsw qkellseilg qkdikiehfg vveqrlnhkk vli1lldvdn
301 leflktlvkg aewfgsgsri ivitqdrqll kaheidlvye vklpsqglal kmisqyafgk
361 dsppddfkel afevaelvgs lplglsvlgs slkgrdkdew vkmmprrlnd sddkieetlr
421 vgydrlnkkn relfkciacf fngfkvsnvk elleddvglt mlaeesliri tpvgiemhn
481 llekgreid rakskgnpgk rqfltnfedi revltekgt etllgirlph pgyltrrsfl
541 ideksfkkgmr nlqyleigyw sdgvlpqslv yfprklkrlw wdncplkrlp snfkaeylve
601 lrmvnsklek lwdgtqplgs lkkmdlynsy klkeipdls1 ainleelnle ecesletlps
661 siqnaiklre lncwgllid lkslegmcnl eylsvpswss rectqgivfy prklksvlwt
721 ncplkrlpsn fkaeylveli meyseleklw dgtqslgslk emnlrysnl keipdlslai
781 nleeldlfgc vslvtlpssi qnatkliyld msecenlesf ptvfnlksle yldltgcpln
841 rnfpaikmgc awtrlsrtrl fpegrneivv edcfwnknlp agldyldclm rcmpcefrse
901 qltflnvsgc kleklwegiq slgsleemdl sesenlkelp dlskatnlkl lclsgckslv
961 tlpstignalq nlrrlymnrc tglevlptdv nlssletldl sgcsslrfp listnivcly
1021 lentaieeip dlskatkles lilnnckslv tlpstignalq nlrrlymnrc tglellptdv
1081 nlssletldl sgcsslrfp listriecl y lentaieevp cciedftrlt vlrmyccqrl
1141 knispnifrl tsatlafdt crgvikalasd atvvatmedh vscvplsni eytcerfwda
1201 csdyysddfe vnrnpirlst mtvndvefkf ccsitikecg vrllyvyqet ehnqqttrsks
1261 krmrrmtsgts eedinlpygq ivadtglaal ntelslggge assstslege alcvddymin
1321 eeqdegipil ypvydiddm wrslffgdtd ddmwrslysa e
//
```

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Aug 15 2006 13:27:38



## Blast 2 Sequences results

PubMed

Entrez

BLAST

OMIM

Taxonomy

Structure

### BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.14 [May-07-2006]

Matrix BLOSUM62 gap open: 11 gap extension: 1

x\_dropoff: 50 expect: 10.000 wordsize: 3 Filter  View option Standard

Masking character option X for protein, n for nucleotide Masking color option Black

Show CDS translation Align

**Sequence 1:** gi|6449046|gb|AAF08790.1|downy mildew resistance protein RPP5 [Arabidopsis thaliana]  
Length = 1361

**Sequence 2:** gi|109946537|gb|ABG48447.1|At1g75250 [Arabidopsis thaliana]

>gi|15222161|refNP\_177661.1| transcription factor [Arabidopsis thaliana]

>gi|41618978|gb|AAS09995.1| MYB transcription factor [Arabidopsis thaliana]

>gi|10092271|gb|AAG12684.1| myb-related protein; 20671-21051 [Arabidopsis thaliana]

Length = 126

**No significant similarity was found**

CPU time: 0.03 user secs. 0.01 sys. secs 0.04 total secs.

Lambda K H  
0.319 0.136 0.404

Gapped  
Lambda K H  
0.267 0.0410 0.140

Matrix: BLOSUM62  
Gap Penalties: Existence: 11, Extension: 1  
Number of Sequences: 1  
Number of Hits to DB: 1008  
Number of extensions: 700  
Number of sequences better than 10.0: 0  
Number of HSP's gapped: 0  
Number of HSP's successfully gapped: 0  
Length of query: 1361  
Length of database: 1,358,419,857  
Length adjustment: 147  
Effective length of query: 1214  
Effective length of database: 1,358,419,710  
Effective search space: 1649121527940  
Effective search space used: 1649121527940  
Neighboring words threshold: 9  
X1: 16 ( 7.4 bits)  
X2: 129 (49.7 bits)  
X3: 129 (49.7 bits)  
S1: 41 (21.8 bits)

**Exhibit I**

S2: 85 (37.4 bits)